

STUDIES ON 'BALANCED FOOD'

III. Nutritive Value of the Food

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ABSTRACT

The nutritional value of a 'balanced food' processed in these laboratories has been assessed by studying (a) the supplementary value of the food to a poor rice diet, (b) the value of the food as a full ration, (c) the growth and reproduction over two generations, (d) the availability of calcium and phosphorus, and (e) the availability of B-complex vitamins. Based on the data obtained in these investigations, it has been concluded that the food would be of considerable benefit, particularly to those sections of population consuming a poor diet.

In the previous communication¹, the quality of the proteins of the balanced food was studied by four different methods. The data showed that the food could meet the protein needs of people consuming a poor diet where either the quality or the quantity of proteins was inadequate. It was felt necessary to collect further information on the nutritive value of the food. The observations collected from these studies are presented here.

Supplementary value of the food to a poor rice diet:—The growth of young albino rats fed a 'poor rice diet'² in which 25% of the rice was replaced by the 'balanced food' was taken as the measure of the supplementation. The enriched and non-enriched foods were used for the studies. American multi-purpose food was taken as the basis for obtaining a comparison. There were thus four groups of young albino rats, four to five weeks old and weighing 40 to 60 gms. The animals were distributed into the groups with due consideration to their age, weight, sex and littermate.

The calculated quantity of the diets in each case was cooked and divided equally among the different animals in the groups. During the experimental period of eight weeks, the rats were weighed every week. The data on average increase in body weight and weight gain per gram of food consumed are presented in Table I.

The different supplementary foods have produced substantial increases of nearly 3 times. The American food and the processed enriched food

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have given almost the same order of response, showing thereby that as far as the supplementary value is concerned there is practically no difference between these two foods. The non-enriched food has also given a fairly high value, but is not as good as the other two foods. This may be due to the lack of vitamins and minerals in the non-riched food.

TABLE I
Supplementary value of the processed foods to the poor South Indian diet

Group	Initial weight (g)	Final weight (g)	Gain in weight (g)	Average weekly increase in wt. (g)	Average weekly food intake (g)	Average weight gained per gm. food intake (g)
P.S.I.D.	47.4	83.6	36.2	4.52 ± 0.52	71.8	0.063 ± 0.001
American M.P.F.	47.2	164.8	117.6	14.8 ± 2.5	85.8	0.170 ± 0.008
Balanced food A	46.4	164.0	117.6	14.7 ± 1.2	87.6	0.168 ± 0.005
Balanced food B	47.0	145.4	98.4	12.3 ± 1.0	83.5	0.147 ± 0.002

Value of the 'balanced food' as a full ration:—Although the supplementary value of the food has been found to be satisfactory, it was felt desirable to study the effect on growth of rats when the food is used as the full ration.

The comparison in this case was against the stock diet used in the animal house of this Institute. The enriched food was taken up for the study. The non-enriched food was not included as it was felt that lack of adequate vitamins would mitigate against normal growth, particularly when fed over a long period.

There were two groups of rats, each group containing nine animals, three males and six females, the distribution being on the usual basis. This experiment was continued after eight weeks for the two generation studies. It was for this purpose that nine rats were taken in each group. One group received the laboratory stock diet and the other the enriched 'balanced food'. Two drops of Adexolin and Viteolin were given by mouth to each rat twice a week. The rats were weighed every week during the experimental period of eight weeks. Data on rate of growth, food consumption and growth per gram of food consumed are given in Table II.

TABLE II
Effect of feeding the balanced food as a full ration

Group	Initial weight (g)	Final weight (g)	Gain in weight (g)	Average weekly increase in wt. (g)	Average weekly food intake (g)	Average weight gained per gm. food intake (g)
Laboratory stock diet	49.7	146.0	96.3	12.0 ± 3.5	83.5	0.144 ± 0.009
Balanced food A	49.7	144.1	94.4	11.8 ± 3.6	83.1	0.141 ± 0.009

The results show that as compared with the laboratory stock diet the balanced food' has produced satisfactory growth. It may, therefore, be stated that the food has no deficiency in any of the essential nutrients required for optimum growth nor any deleterious effects when taken as a sole source of ration.

Two generation studies:—The earlier two trials were of comparatively short duration, the experimental period being eight weeks. In testing the influence of consuming foods of this type, it is important to determine the influence of fairly long term feeding. Hence the rats in the previous experiment were continued on the two respective diets, *viz.*, laboratory stock diet and balanced food. When the animals reached the age of $3\frac{1}{2}$ months, they were mated, using in each group two females to one male. There were thus three sets of mating cages in each group.

As and when litters were born, the young ones were counted, weighed and put in a separate cage along with the mother. All the females in both the groups (except one in each group) gave litters in about a month's time from the date of mating.

The number of young ones and their weight at the weaning stage were recorded. All the litters from the same group were mixed and a random selection of six females and three males was made. This represented the second generation and the experiment was continued in the same manner till litters were weaned. The results are presented in Table III.

TABLE III
Two generation studies with laboratory stock diet and balanced food A

Group	Number of rats	Number of sterile rats	Litter strength	Number of young ones survived	Percentage of young ones survived
First generation					
Balanced food A	6	1	6	4.8	82.6 ± 5.5
Laboratory stock diet	6	1	6.8	5.6	85.2 ± 4.4
Second generation					
Balanced food A	6	—	6.3	5.2	84.8 ± 4.5
Laboratory stock diet	6	1	6	4.8	82.0 ± 4.5

The average weights of litters at birth and at weaning age in the control and experimental groups were 5 and 32 g. respectively. The rats in both the groups appeared quite healthy and active during the period of study.

The above data show practically no difference between the two types of diets over the two generation period. The number of litters, the number survived and the birth and weaning weight are all nearly the same for both the groups.

Continued consumption of the food has not led to sterility in the females. When the food is consumed as the sole source of ration, no adverse effects are likely to be developed by the people.

Availability of calcium and phosphorus:—The principal deficiency in the 'poor South Indian diet' is in its calcium content. Growth of rats on this diet is very considerably improved by the mere addition of calcium or of other supplements containing calcium. The availability of calcium in a food material is often governed by the presence or otherwise of certain materials. Thus excessive amounts of oxalate adversely affect the availability of calcium. Fische and Sherman³ found the high oxalate content of spinach to interfere with utilisation of the calcium. Mellanby⁴ observed that a diet rich in cereals exerted a deleterious effect on the calcification of bones and teeth. Bruce and Callow⁶ traced the anticalcifying action of cereals to lowered calcium availability as a result of calcium binding itself with phytin to form calcium-phytate.

Giri⁷ also found a large part (about 50-70%) of the phosphorus contained in the commonly used cereals in India to exist as phytin phosphorus and therefore stated that the phosphorus in these materials was not fully available. Tubers, green and leafy vegetables were found to contain very little phytin phosphorus. The availabilities of calcium and phosphorus in rice, ragi, cambu and cholam were also studied. Cambu and cholam were found to be better sources than rice and ragi.

McCance *et al*⁸ attributed increased availability of calcium on a high protein diet to the amino acids which solubilised the calcium and thereby favoured absorption.

Givens and Mendel⁹ recorded that fatty acids utilised to a less extent tended to increase the faecal calcium. Basu and Nath¹⁰ found mustard, sesame and groundnut oils as well as butter to improve calcium absorption in humans.

Basu *et al*¹¹ observed that calcium ingested during the chewing of betel leaves was well absorbed. Basu and Ghosh¹² reported that the availability of calcium in cabbage was of the same order as that of milk.

Experiments were carried out to determine the extent of availability of the calcium and phosphorus in the 'balanced food'. The enriched food employed in these studies contained only the vitamins, but not the minerals, calcium and iron. It was of interest to find out the availability of calcium already present as also the influence on its availability, if any, brought about by extra vitamins. Extra calcium as calcium lactate could normally be expected to be fully available. The non-enriched food was supplemented to poor South Indian diet and the calcium availability under such conditions was evaluated.

Albino rats weighing 130 to 150 gms* were employed for the study. The rats were given the unenriched food (B) for seven days and urinary and faecal collections were made during the last four days of this period, the first three days

* Generally adult rats are employed for such studies.

being the time for adjustment. After the seventh day, the rats were rested on stock diet for two days and put on the enriched food (*i.e.*, with added synthetic vitamins only) for seven days. Urinary and faecal collections were made during the last four days. The rats were again rested on the stock diet for two days and were then given the poor South Indian diet for seven days. With a rest of two days again on stock diet, they were given the poor South Indian diet supplemented with the food B to the extent of 25%. The urinary and faecal collections were made during the last four days in the above two cases as well.

The faeces from the individual rats were separately stored in a frigidaire and when taken up for analysis they were dried, weighed and powdered. Aliquots were taken for duplicate analysis of calcium and phosphorus. Calcium in faeces and diet was determined by the procedure outlined by McCrudden.¹³ The urine samples were evaporated to dryness on the water-bath and ashed in a muffle furnace. The residue was dissolved in hydrochloric acid. Calcium precipitated as calcium oxalate was titrated in the usual manner against standard potassium permanganate. Phosphorus in the urine (total and inorganic) as also faeces and diet was determined by King's modification¹⁴ of Fiske and Subba Rao's method.¹⁵

The total amount of phosphorus and calcium ingested during the metabolism studies was calculated by collecting the unconsumed diet everyday and

TABLE IV
Availability of calcium and phosphorus

Mineral	Total food intake (gm.)	Total mineral intake (mg.)	Excretions		Mineral retained (mg.)	Availability (%)
			Faecal (mg.)	Urinary (mg.)		
Balanced food B - Ca/P = 0.77						
Calcium	51.2	161.7	25.3	2.07	134.3	83.2 ± 1.04
Phosphorus	51.2	204.0	64.9	28.0	111.0	54.4 ± 0.86
Vitamin enriched balanced food B - Ca/P = 0.77						
Calcium	60.4	190.1	31.8	1.4	158.2	82.0 ± 1.03
Phosphorus	60.4	241.5	80.9	34.3	126.3	52.3 ± 1.02
Poor South Indian diet - Ca/P = 0.37						
Calcium	61.7	24.19	12.4	0.77	11.02	45.5 ± 0.46
Phosphorus	61.7	65.4	17.9	24.3	23.2	35.2 ± 0.61
75% poor South Indian diet + 25% balanced B - Ca/P = 0.51						
Calcium	69.2	72.6	31.6	1.2	39.2	54.8 ± 0.38
Phosphorus	69.2	142.5	36.2	26.4	79.9	54.1 ± 0.56

making allowance from the total food supplied. Thus the calcium intake was arrived at. The observations are recorded in Table IV.

The availability of calcium in the "Balanced food" is fairly high. The value obtained is higher than the calcium availabilities reported for some of the cereals (Giri, *loc cit.*). The results suggest that the availability of calcium is nearly as good as that of milk which is generally considered to be a rich source of this mineral.

The results do not show any extra benefit by the addition of vitamins to the 'balanced food' on calcium availability.

Phosphorus availability data show nearly the same trend as that of calcium. The values agree very closely with the availability of phosphorus in some cereals.

Availability of B-complex vitamins:—The part played by vitamins in the nutrition and the necessary for ensuring an adequate supply of these accessory food factors in the diet are too well known and therefore it is not necessary to discuss these aspects here. One object of processing a 'balanced food' was to provide the consumers of poor diet a fairly good source of some of the B-complex Vitamins and Vitamin A. Vitamin A was completely made up from synthetic source and as such there would be no necessity for finding out its availability. A part of the B-complex vitamins was also added as synthetic vitamins. But a good part was already present in the different oil-cake flours used in the food. As such it was of importance to determine the extent to which these would be available. The overall growth response, when fed to rats depleted of their stores of B-complex vitamins, was followed.

Twenty-four young albino rats, about 30 days old and weighing 40 to 50 gms. were fed a B-complex-free diet adequate in other respects. Casein was rendered vitamin free¹⁶ and used as the source of protein in the diet during the depletion period. The animals registered a slight increase in weight during the first week owing probably to the stores of B-complex vitamins in the body, but showed a decrease in the second week. At this stage, the rats were divided into four groups consisting of six rats in each group on the basis of their weight and sex. One group, serving as the control, was continued on the same diet. The second group received the 'American Multi-purpose Food' supplement. The third and the fourth groups were given the 'balanced food' supplements, the compositions A and B, in which A had extra synthetic vitamins, while B had only the vitamins already present in the oil-cake flours. The different food supplements formed 50% of the diet.

The feeding with the experimental diet was conducted for four weeks, and the individual rats were weighed every week. The first group of rats, which were continued on the vitamin-free casein diet, died between the third and fourth week from the commencement indicating thereby that the diet fed was deficient in B-complex vitamins.

The weights of the three groups of animals under the experimental diets during the period of the experiment are given in Table V.

TABLE V
Extent of the availability of the B-complex vitamins in the balanced foods

Group	Average initial weight (g)	Average weight at the end of			
		1st week (g)	2nd week (g)	3rd week (g)	4th week (g)
American MPF	45.3 ± 0.9	63.3 ± 0.8	84.5 ± 1.3	100.0 ± 1.9	110.8 ± 2.3
Balanced Food A	46.3 ± 1.4	60.5 ± 1.6	76.5 ± 2.7	92.4 ± 2.2	101.2 ± 2.9
Balanced Food B	45.3 ± 1.0	58.8 ± 1.6	69.0 ± 4.0	79.0 ± 3.8	84.0 ± 3.7

The results presented in Table V indicate that the B-complex vitamins present in the different oil-cake flours are not readily available. The non-enriched formula (B) has given the least increase in weight. Even food A has not come up to the standard of the American food from the point of view of availability of B-complex factors. The reason is quite obvious. In the American food the entire quantity of B-complex vitamins has been added as synthetic vitamins, whereas in balanced food A, only a part of these vitamins has been incorporated as synthetic vitamins. Naturally, therefore, the availability has been limited. Even so, the growth response, though less than what has been obtained with the American food, may not be considered to be very poor.

A consideration of the results of the earlier and the present studies would show that the supplementary food processed in these laboratories can be a very useful source of proteins, minerals and vitamins.

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REFERENCES

1. Lal, B. M. and Rajagopalan, R. . . *J. Indian Inst. Sci.* 1957, **39**, 169.
2. Aykroyd, W. R. and Krishnan, B. G. *Indian J. Med. Res.*, 1937, **25**, 367.
3. Ficke, M. L. and Sherman, H. C. . . *J. Biol. Chem.*, 1945, **110**, 421.
4. Mellanby, E. . . *Report Med. Res. Council (London)*, 1921, No. 61.
5. Idem . . . *Ibid*, 1925, No. 93.
6. Bruce, H. M. and Callow, R. K. . . *Biochem. J.*, 1934, **28**, 517.
7. Giri, K. V. . . *Indian J. Med. Res.*, 1940, **28**, 101.
8. McCance, R. A., Widdowson, E. M. and Lebman H. *Biochem. J.*, 1942, **36**, 686.
9. Givens, M. H. and Mendel, L. B. . . *J. Biol. Chem.*, 1917, **31**, 441.
10. Basu, K. P., and Nath, H. R. . . *Indian J. Med. Res.*, 1946, **34**, 27.

11. Basu, K. P. Basak, M. N. and De, H. N. *Ibid*, 1942, 30, 309.
12. Basu, K. P. and Ghosh, D. .. *Ibid*, 1943, 31, 9.
13. McCrudden, F. H., .. *J. Biol. Chem.*, 1911-12, 10, 187.
14. King, E. J. .. *Biochem. J.*, 1932, 32, 192.
15. Fiske, C. H. and Subba Rao, Y. .. *J. Biol. Chem.*, 1925, 66, 373.
16. Guha, B. C. and Drummond, J. C. .. *Biochem. J.*, 1929, 23, 880.